ANNUAL REVIEWS Further
Quick links to online content

Ann. Rev. Phytopathol. 1987. 25:293–315
Copyright © 1987 by Annual Reviews Inc. All rights reserved

FUNGAL ENDOPHYTES OF GRASSES

M. R. Siegel

Department of Plant Pathology, University of Kentucky, Lexington, Kentucky 40546-0091

G. C. M. Latch

Department of Scientific and Industrial Research, Palmerston North, New Zealand

M. C. Johnson

Department of Biological Sciences, Georgetown College, Georgetown, Kentucky 40324

INTRODUCTION

The term endophyte (Greek: *endo = within + phyte = plant*) has been defined as an organism contained or growing (entirely) within the substrate plant, whether parasitically or not (100, 106). Using this term in its broadest sense, the subject of this chapter could include all fungi that spend all or nearly all of their life cycles in the host grasses. We do not discuss such endophytic fungi as the smuts and vesicular-arbuscular mycorrhizas that infect grass. Instead, our interests lie primarily with a specific group of clavicipitaceous fungi that belong or are related to fungi in the tribe Balansiae (5, 24). These fungi either are true endophytes that never produce external fructifications on the plant or else may produce external mycelium and/or spores that affect flower and seed production. We focus specifically on those grass–endophyte complexes that may also cause maladies of grazing animals.

Using the above criteria, two recent events involving grass–endophyte associations have important implications for the livestock industry. In 1977 Bacon et al (9) reported the close association of an endophyte *(Sphacelia typhina)* in infected tall fescue *(Festuca arundinacea)* and the incidence of

0066-4286/87/0901-0293$02.00

fescue toxicosis (summer syndrome) in cattle. A similar association between a then unidentified endophyte in perennial ryegrass *(Lolium perenne)* and the incidence of ryegrass staggers in sheep in New Zealand was reported in 1981 by Fletcher & Harvey (27).

Since the initial work of Bacon et al (9) in 1977, researchers have come to further understand the relationship between fungal endophytes of grasses and animal toxicoses. This understanding includes the origin and incidence of infected grasses, modes of dissemination of the fungi, identification of the chemicals responsible for toxicoses, and control of the fungi. Specifically, what has been learned is that endophyte-infected tall fescue and perennial ryegrass are widely distributed in the United States and New Zealand, respectively; endophytic fungi are only seed disseminated; specific chemicals are responsible for fescue toxicosis and ryegrass staggers; infected pasture grasses cost the livestock producer hundreds of millions of US dollars annually in lost production; the fungi cannot be controlled in the field, but endophyte-free cultivars do result in greatly improved livestock production in the United States; and lastly, the relationship between grass and symbiont is primarily mutualistic, resulting in many benefits to host and fungus (5, 10, 97). With regard to this final point, it is now recognized that these grass endophytes can play an important role in survival of the host plants subjected to environmental stresses (insects, grazing animals, drought, and heat). The potential for improved insect resistance of endophyte-infected grasses has already been exploited by the selection of naturally infected cultivars of *Festuca* and *Lolium* species (29, 30).

This chapter, therefore, reviews the harmful and beneficial effects of endophytic fungi on forage and turf grasses and on grazing animals. It includes a historical perspective on the fungi (incidence, dissemination, and control), the relationship between infected grasses and animal toxicoses, the nature of the grass–fungus interaction, and the value of infected and noninfected cultivars in pasture and turf.

HISTORICAL PERSPECTIVES

The presence of endophyte mycelium in the seed of a grass *(Lolium temulentum)* was first recorded by Vogl in 1898 (105), but the earliest known specimen of this host and fungus dates back 4,400 years to seeds found in the tomb of a Fifth Dynasty Egyptian Pharoah (64). Vogl's (105) discovery aroused interest because seeds of *L. temulentum* were known to be toxic to animals, and there was speculation that the fungus was responsible. Other species of *Lolium* were also found to contain mycelium in a high proportion of their seeds; thus, the endophytic nature of the fungus in plants was established (28, 76).

Classification

Endophytes of grasses and sedges are grouped in the tribe Balansiae of the Clavicipitaceae. Within this tribe are the genera *Balansiopsis, Atkinsonella, Myriogenospora, Balansia,* and *Epichloe* (24, 66). The taxonomy of endophytes, for which no teleomorphic state has been found, is still being debated. Morgan-Jones & Gams (70) created the section *Albo-lanosa* in the genus *Acremonium* to accommodate the endophyte commonly found in tall fescue. They named this fungus *A. coenophialum,* and subsequently, endophytes in other species of *Festuca* and *Lolium* have been placed in this section (59, 116, 117). These endophytes may be related to the clavicipitaceous teleomorph *Epichloe* (59, 70), but they have temporarily or permanently lost the ability to form sexual spores. Rykard et al (91) argue that it would be preferable to leave these newly named *Acremonium* species in the genus *Sphacelia,* the conidial state of *Epichloe typhina.* This question will only be resolved when the teleomorphic states of the fungi are found.

Latch et al (59) have described two seed-borne endophytes in perennial ryegrass and tall fescue with *Gliocladium*-like and *Phialophora*-like conidia, respectively. The relationship of these endophytes to those in the Balansiae is unclear.

All these Balansiae, except for *Myriogenospora,* produce systemic infections in leaves, culms, and inflorescences, but most become conspicuous only when sporulation occurs as the plant flowers. Many endophytes prevent their host from flowering, whereas others do not interfere with the plant's reproduction, and indeed, their mycelium may be present in the seed. Intercellular hyphae have been found in the ovary wall and throughout the nucellar tissue of *F. versuta,* tall fescue, and perennial ryegrass ovules (18, 65, 78). However, in some perennial ryegrass megagametophytes, hyphae of *Acremonium lolii* (59, 61) become intracellular when they breach the embryo sac and enter the antipodal cells (78). As the embryo enlarges and differentiates, the hyphae pass into the shoot apical region. Thus, some endophytes are very efficiently dispersed with their host seed and apparently have no need for spores as a means of dissemination. Indeed, it is believed that the only means of dissemination of *Acremonium* endophytes is through maternal transmission in infected seed (4, 54, 96).

Incidence

Species of *Balansia, Balansiopsis, Atkinsonella,* and *Myriogenospora* infect a wide range of grasses commonly found in the tropics that have a C_4-type photosynthetic pathway. Most of these grasses are regarded as weeds or as having minor agricultural importance (24, 66). *Epichloe typhina* is found on important genera of forage and turf grasses such as *Agrostis, Dactylis, Festuca, Holcus, Hordeum, Lolium,* and several other genera of minor agricultural importance. The striking choke symptom of mycelium around the inflorescence is present on most infected plants (24, 92).

Endophytes that have *Acremonium, Sphacelia, Gliocladium*-like, or *Phialophora*-like anamorphic states and that produce no external fructifications on grasses were originally found only in species of *Festuca* and *Lolium* (9, 59, 70). Recent searches have extended the host range to include species of *Bromus, Poa,* and *Stipa* (111, 115, 117). Undoubtedly, further searches will extend this host range.

The incidence of endophytes in tall fescue, perennial ryegrass, and Italian ryegrass *(Lolium multiflorum)* has been studied because these grasses are of prime importance in many countries. Tall fescue is grown on 12–14 million hectares in the United States, and the two ryegrasses are the main pasture grasses sown in Europe and New Zealand. Endophytes have been found in seeds of these three grasses collected from areas of Europe where the species are indigenous and may possibly have originated (61, 96). The seed-borne nature of endophytes has resulted in their being spread, along with their hosts, throughout the world. Over 90% of tall fescue pastures in the United States contain plants infected with *Acremonium coenophialum* (97, 108). A survey by the Auburn University fescue diagnostic laboratory of samples from twenty-six states showed that 94% of pastures contained endophyte, and the plants in these pastures had a mean infection of 58% (95). The dominant cultivar of tall fescue in the United States is Kentucky-31, and most plants are infected with endophyte (96); however, new cultivars with a low incidence of endophyte have now been developed (97).

Seed samples from 53 of 64 old pastures of perennial ryegrass from eight European countries were found to be infected with the endophyte *A. lolii* (61). Recently sown pastures probably have a much lower incidence of endophyte because a survey of sixteen current European cultivars showed that only four contained endophyte, the average incidence being 15% in these cultivars (61). In New Zealand, the majority of plants in seven million hectares of perennial ryegrass are infected with *A. lolii,* and the two most commonly sown cultivars have 65–95% of the plants infected (54, 94, 97). There is little information on the incidence of an *Acremonium*-like endophyte in Italian ryegrass pastures. In Europe it appears to be low, especially among recent cultivars, but in New Zealand over 50% of plants of new Italian ryegrass cultivars may be infected with this endophyte (57, 61).

Methods of Detection

Grasses infected with endophytes that have as their anamorphic state *Acremonium, Gliocladium*-like, or *Phialophora*-like conidia are symptomless. The mycelium is intercellular within the host plants, but several techniques are available for detection and identification of these endophytes.

STAINING OF MYCELIUM Grasses infected with endophytes have hyphae in the leaf sheaths. If the adaxial epidermis is removed and stained, the septate, intercellular mycelium can be seen with the aid of a light microscope (16, 55). Hyphae of *Acremonium* spp. and *E. typhina* run parallel to the leaf-sheath axis and are seldom branched, whereas hyphae of the *Gliocladium*-like and *Phialophora*-like endophytes are branched profusely and present in greater quantities than those of the *Acremonium* spp. (59). Most of the endophytes can be found in all above-ground parts of the plant, and hyphae of the *Gliocladium*- and *Phialophora*-like spp. are also present in roots (59). Some endophytes prevent seed formation, but with others, viable seeds are produced that contain a dense mat of mycelium in the region of the aleurone layer (28, 69). This mycelium can be detected by softening seeds with sodium hydroxide or nitric acid, followed by degluming, squashing, and staining for microscopic examination (16). Unfortunately, the technique does not indicate whether mycelium is viable. In order to demonstrate endophyte viability, seeds must be germinated and the 3–4-week-old seedlings examined for mycelium (59, 108). A staining technique that distinguishes between living and dead mycelium in seeds would be of great value.

SEROLOGY Antisera have been prepared to cultures of *A. coenophialum* (48) and *A. lolii* (72) and used in an enzyme-linked immunosorbent assay (ELISA) to detect antigens of the fungi in tall fescue and perennial ryegrass. ELISA based on antibodies produced against mycelia of these two fungi does not differentiate serologically between *A. coenophialum, A. lolii, Acremonium*-like spp. in Italian ryegrass, and *E. typhina* (49, 74; D. R. Musgrave, personal communication). Musgrave et al (74) suggest that the major soluble antigen is a polysaccharide moiety of a protein lipopolysaccharide complex. They speculate that purified soluble antigens could be used to produce specific antisera for each of the endophytes. The ELISA technique is particularly useful for determining the location and quantity of mycelium in infected plants and for detecting endophyte mycelium in seeds (44, 72). However, difficulties experienced in using the technique have led to a decrease in its use for endophyte detection, and most endophyte diagnostic laboratories now use the staining technique (95, 108, 109). ELISA does not differentiate between viable and nonviable mycelium, so seed must be germinated and the young plants tested by ELISA or stained to verify the viability of mycelium in seed.

CULTURE Fungal endophytes of grasses can be cultured on artificial media, and most have been induced to sporulate. In some plant tissues, and especially in seeds, it is difficult to identify endophytes in situ, and so it is necessary to culture the fungi. Many endophytes grow very slowly in culture and are easily overrun by contaminating fungi and bacteria. Hence, culturing endophytes from infected plant tissues is not used as a routine method of endophyte detection, but rather as an adjunct for endophyte identification. It is relatively easy to isolate and culture endophytes from leaf sheaths, nodes, and stem pith (9, 16, 55, 116), but isolating endophytes from seeds is more difficult, especially from seeds heavily contaminated with saprophytic fungi. The method most commonly used is to surface-sterilize seeds in sulfuric acid (59, 108), but even this drastic treatment does not remove all fungi from heavily contaminated seeds. There is evidence (108) that this treatment kills the endophyte in some seeds. Thus, a more satisfactory surface-sterilization procedure is required.

INSECT BIOASSAY When given the choice of endophyte-infected and endophyte-free grass, some species of insects will feed only on the endophyte-free grass (31, 47, 58, 86). A relatively clear-cut preference for endophyte-free tall fescue and perennial ryegrass was shown by several species of aphids (47, 58). This finding led Latch et al (58) to advocate the use of *Rhopalosiphum padi* as a bioassay to detect tall fescue plants infected with *A. coenophialum* for large-scale, rapid screening of this endophyte in seedlings and mature plants.

Methods of Control

As previously mentioned, it is thought that endophytes of ryegrass and tall fescue are transmitted only through seed and that infection of endophyte-free grasses does not occur in nature (96, 119). Hence, if endophyte mycelium in seeds can be killed without harming the seed, or if mature infected plants can be freed from endophyte infection, then these plants will remain free of endophyte and will set endophyte-free seed. Several methods of killing endophytes in seeds and plants have been devised.

STORAGE OF SEED Viability of *A. lolii* and *A. coenophialum* mycelium in seed declines during storage (55, 75, 98). Neill (75) found that endophyte in perennial ryegrass seed was dead after two-years storage at ambient temperature, but it was shown subsequently that endophytes in perennial ryegrass seed remained viable for at least fifteen years if stored at low temperature (0–5°C) and low humidity (25–50%) (54). Recent studies have quantified the effect of environmental conditions and demonstrated that low temperature and low seed-moisture content will preserve endophyte viability (88, 108). If it is necessary to kill the endophyte but not greatly harm seed viability, the seed should be stored at high temperatures (30°C) and at a seed-moisture content of 8–10%.

HEAT Soaking seeds of perennial ryegrass and tall fescue in hot water for short periods of time killed the endophytes within them, but seed germination was reduced by up to 50% (55, 98, 118). A complete kill of endophyte with only a minor reduction in seed viability was obtained when tall fescue seed was kept in hot moist air (49°C) for seven days (98).

CHEMICAL Endophyte mycelium in seeds of perennial ryegrass and tall fescue has been killed by applying fungicides that inhibit ergosterol biosynthesis, such as propiconazole, prochloraz, and triadimefon, to seeds (55, 98, 119). High application rates of fungicides are required for complete control of endophytes, and some stunting and death of seedlings are inevitable. Higher rates of fungicide application are required to kill mycelium of *A. coenophialum* in seed of tall fescue than those needed for *A. lolii* in perennial ryegrass. These fungicides have been less effective in controlling endophytes growing in plants. Repeated foliar applications of triadimefon to plants grown in a greenhouse reduced the amount of *A. coenophialum* mycelium in tall fescue, but did not eradicate the fungus (98, 119). Granules of propiconazole applied to the soil in pastures of tall fescue suppressed the endophyte for many months, but did not eradicate it (119). Complete eradication of endophyte in perennial ryegrass and tall fescue has been accomplished by growing plants in sand and drenching benomyl around the roots (55; G. C. M. Latch, unpublished data), and in tall fescue by using bitertanol or propiconazole granules and foliar sprays (98, 119). With the fungicides available at present, it is clear that eradication of endophytes from infected pastures is not economically feasible. There is, however, a great need for a fungicide that could inexpensively accomplish that purpose.

Plants and seeds from which endophytes have been eliminated by any of the three methods just described will produce endophyte-free seed that can then be used as mother stock to build up endophyte-free lines. Thus, the simplest and most effective way of controlling animal disorders that result from grazing endophyte-infected grass is to establish pastures from endophyte-free seed. In the United States several new cultivars of tall fescue have recently been released and certified to have fewer than 5% of seeds infected with *A. coenophialum* (10, 44, 109).

ANIMAL TOXICITIES

As indicated in the introduction, renewed interest in endophyte-infected grasses began with a few key investigations into causes of animal maladies, specifically fescue toxicosis and ryegrass staggers (9, 27). Losses due to these disorders, in terms of animal production, have been estimated to be in the hundreds of millions of US dollars annually (82; J. A. Boling, personal communication). However, these estimates do not include losses in reproduction of cattle that may also be associated with these livestock disorders (36). Reproductive capacity of both female and male mammals appears to be decreased on diets high in endophyte-infected forage or seed (77, 104, 121–123).

Fescue Toxicosis

The poor cattle performance associated with *A. coenophialum*–infected tall fescue is characterized by reduced weight gain, decreased milk production, lower feed intake, rough haircoat, excessive salivation, increased respiration rate, and high rectal temperatures (40). These symptoms most often occur in, but are not limited to, the summer months. An additional malady of cattle associated with endophyte-infected tall fescue in some localities is characterized by lameness, ischemic necrosis, dry gangrene of the extremities, and, in severe cases, loss of hooves (35, 40). This latter malady is known as fescue foot and occurs most frequently during the winter, with the onset of clinical symptoms often linked to a sudden drop in ambient temperature.

Several alkaloids have been suggested to contribute to fescue toxicosis. The loline alkaloids, *N*-acetyl and *N*-formyl, have been shown to reach concentrations as high as 0.8% per unit dry weight in stems, leaves, and seeds of endophyte-infected tall fescue and have not been detected in endophyte-free tall fescue plants (15, 50). It has not been established if the loline alkaloids play a role in fescue toxicosis. However, these alkaloids do appear to serve as insect-feeding deterrents (45, 47).

The ergot alkaloids, clavine and ergopeptide types, are produced in vitro by *A. coenophialum* (5, 67, 80) and have been found in low concentrations (relative to the loline alkaloids) in leaf blades and sheaths of endophyte-infected tall fescue collected from pastures (67, 120). Attention has been primarily focused on ergovaline, an ergopeptide alkaloid that constitutes 10–50% of the total ergot alkaloid concentration in the forage (5, 67). Many of the symptoms of fescue toxicosis are consistent with signs of ergot poisoning (5, 68, 120).

Ryegrass Staggers

Perennial ryegrass staggers is a neurological disorder of animals that most commonly affects sheep, but also occurs in cattle, horses, and deer (71). Animals with this disorder appear normal until disturbed, at which time they suffer severe muscular spasms, usually resulting in collapse followed by an apparently quick recovery (71, 97). In severe outbreaks, deaths from falls or drowning are common (71). Ryegrass staggers occurs during the summer and autumn and is commonly associated with warm ambient temperatures and with grazing of short-grass swards (71). In addition to the staggers condition, animals grazing *A. lolii*–infected perennial ryegrass show reduced weight gains, which are probably attributable to reduced intake of herbage (26).

The prime suspect causative agents of ryegrass staggers are the lolitrem neurotoxins (32–34). The major lolitrem neurotoxin, lolitrem B, is a lipophilic complex substituted–indole compound with a molecular weight of 685 and formula $C_{42}H_{55}NO_7$ (84). The lolitrems have not yet been reported to be produced by *A. lolii* in culture, but related tremorgenic toxins, penitrems, janthitrems, and aflatrem, are of fungal origin (34).

Toxicities Attributed to Balansia-*Infected Weed Grasses*

Many warm-season perennial weed grasses infected with species of *Balansia* may possibly be involved with some outbreaks of animal toxicosis (5, 6). Weed grasses parasitized by *Balansia* species include smutgrass *(Sporobolus poiretii)*, broomsedge *(Andropogon virginicus)*, lovegrass *(Eragrostis hirsuta)*, and panicum *(Panicum anceps)* (5, 6). Bacon et al (5) reported that in the summer months a large percentage of a pasture may consist of a weed grass and that the majority of the weed grass may be infected by *Balansia* spp. Several species of *Balansia* have been shown to produce ergot alkaloids in vitro as well as in vivo (3, 5–8). Furthermore, cultures of *Balansia epichloe* isolated from infected smutgrass depressed serum prolactin concentrations in lactating Holstein cows following oral dosing for three consecutive days (107). Prolactin is a pituitary hormone that is necessary for the secretion of milk and that probably also plays a role in affecting the reproductive capacity of both female and male mammals (107). Depressed serum prolactin levels also occur in animals that have consumed *A. coenophialum*–infected tall fescue forage (12, 40, 42) as well as in sheep that have grazed *A. lolii*–infected perennial ryegrass (26).

GRASS–FUNGUS INTERACTIONS

Parasitic and Mutualistic Symbiosis

The endophytic fungi infecting grasses are biotrophic; that is, they obtain all their basic nutritional needs from living tissue. Furthermore, Lewis (62) considers fungi such as *E. typhina* and those responsible for "symptomless parasitism" as belonging to a group of ecologically obligately symbiotic biotrophs (obligate biotrophs). Lewis defined this group of fungi as "parasitic and mutualistic symbionts with no capacity for free living existence other than as gametes, cysts or as laboratory cultures." The terminology and classification are based on de Bary's (22) original concept of symbiosis: "Those which . . . feed on living organisms, whether plants or animals, are termed parasites. Their relationship with their hosts is that of a common life, a *symbiosis*." de Bary recognized that gradation of symbiosis existed and that parasites could either quickly destroy their host or that parasite and host could mutually and permanently live together and support one another.

According to Lewis's scheme, *E. typhina* and *Balansia* spp. would be considered biotrophic and parasitic endophytes, whereas the *Acremonium* endophytes would be biotrophic and mutualistic symbionts. This scheme of classification of *E. typhina* is not absolute because isolates in some host grasses show gradations of symbiosis. Some isolates act as parasitic symbionts (24, 110), whereas others behave, e.g. the symptomless *Acremonium* endophytes, as mutualistic symbionts (29, 92). Whether a specific symbiotic association is parasitic or mutualistic can only be determined by comparing the fitness of the host and fungus when living independently with their fitness when living in association (63).

The major features of mutualistic symbioses are a lack of destruction of host cells or tissue; nutrient or chemical cycling between host and fungus; enhanced longevity and photosynthetic capacity of cells and tissue under the influence of infection; enhanced survival of the fungus; and a tendency toward greater host specificity than seen in necrotrophic infections (62). Researchers (5, 10, 82, 96, 97) have used this concept of mutualism to suggest that the *Acremonium* endophytes infecting perennial ryegrass and tall fescue enter into a relationship with their respective grasses that benefits both host and fungus. These benefits include enhanced dissemination and survival of the fungus, improved growth for the host plant, and tolerance to herbivore feeding (grazing animals and insects).

Benefits to the Fungus

Detection of endophytes by electron microscopy (25, 41), staining (9, 23, 108), and ELISA (72, 96) clearly indicates that intercellular fungal growth of *Acremonium* spp. and isolates of *E. typhina* is distributed unequally in the plant, with highest concentrations found in the leaf sheaths, seeds, and crowns. These regions can act as "sinks" for the accumulation of soluble nitrogenous and carbohydrate substrates (5). These substrates would have to leak out of the plant cells into the intercellular spaces and fluids for uptake by the endophytes. Whether the fungi induce leakage or possess translocational mechanisms for movement of plant products is unknown. However, reciprocal translocation of carbohydrates between host and pathogen has been reported in Bahia grass infected with *Myriogenospora atramentosa* (99). The nutritional requirements of the fungi in the plant are unknown.

In vitro rates of growth, pH and temperature optima, utilization of carbon and nitrogen sources, and synthesis of specific fungal products are known for some endophytes (3, 9, 20, 53, 59, 114). This information cannot be extrapolated to the in vivo situation, but can be used to compare nutritional, physiological, biochemical, and genetic potential of isolates (10). Clearly, the endophytes benefit from the association with their hosts by nutrition, long-term protection, improved dissemination (via seed), and survival.

Benefits to the Host

GROWTH Perhaps of all the potential benefits to infected grasses, changes in plant physiology and patterns of growth are the most complex and difficult to assess. In studies in Kentucky over a four-year period, Siegel et al (96) did not find differences in forage (dry matter), seed yield, and stand persistence of Kenhy tall fescue in plots that ranged from 7–75% of plants infected with *A. coenophialum*. On the other hand, there are reports suggesting that endophyte-free cultivars do not survive as well as infected ones. In Texas, infected tall fescue that had been grazed produced more forage than plants in noninfected grazed pastures (87). In addition, during the third year of the trial many plants died in two of the three noninfected replicated plots. Funk et al (29) reported that over a seven-year period in New Jersey, endophyte-infected tall fescue turf plants outperformed noninfected turf plants from the standpoint of resistance to crabgrass invasion and recovery from summer drought stress.

The differences in the three reports are difficult to reconcile, but as will be shown, infected perennial ryegrass and tall fescue exhibit striking resistance to herbivores (insects and grazing animals). A combination of environmental stresses (heat and drought), herbivore feeding, and competition with other grass and weed species could account for the differences reported in growth and survival between infected and noninfected grasses. Biotic and abiotic stresses have been reported to be the reason for the inability of endophyte-free perennial ryegrass cultivars to survive in New Zealand (11, 82).

There are no differences in forage quality (crude protein, neutral detergent fiber, acid detergent fiber, and digestibility) between endophyte-infected and noninfected tall fescue (14). When infected tall fescue was grown under controlled conditions, the plants had greater rates of photosynthesis and a greater increase in fresh weight; they produced more tillers during regrowth; and they had a lower percentage leaf roll (during a drying cycle) than did endophyte-free tall fescue. Infected plants also used water more efficiently than did noninfected plants (L. P. Bush, personal communication). These differences would support the hypothesis that infected tall fescue plants have advantages over noninfected plants when grown during periods of environmental stress.

The clearest example of enhanced growth has been reported for *A. lolii*–infected perennial ryegrass grown under controlled conditions in New Zealand (60). Infected plants (when compared to noninfected) had significant increases in total leaf area, tiller numbers, and growth of leaves, but the shoot–root ratio was unaffected.

The chemical basis for the changes in physiology and growth patterns of *Acremonium*-infected plants is unknown. It is possible that members of the

Table 1 Species of insects reported to be affected by endophyte-infected *Festuca* and *Lolium* species of grasses[a]

Insect	Grass	Fungus	Reference
Crambus spp. (Sod webworm)	PRG[b]	A.l.	31
Listronotus bonariensis (Argentine Stem Weevil)	PRG, TF[b]	A.l., A.c.	86, 101
Sphenophorus parvulus (Bluegrass billbug)	PRG[b]	A.l.	2
Spodoptera frugiperda (Fall armyworm)	PRG, TF	A.l., A.c.	38, 39
Acheta domesticus (House cricket)	PRG, TF	A.l., A.c.	1
Rhopalosiphum padi (Oat-birdcherry aphid)	TF	A.c.	47, 58
Schizaphis graminum (Greenbug aphid)	PRG, TF, HF[c], CF[c]	A.l., A.c., E.t., E.t.	47
Oncopeltus fasciatus (Milkweed bug)	TF	A.c.	47
Blissus leucopterus hirtus (Chinch bug)	CF[b]	E.t.	29
Heteronychus arator (Black beetle)	PRG[b]	A.l.	82
Draculacephala antica (Sharpshooter leafhopper)	TF[b]	A.c.	52
Chaetocnema pulicaria (Corn flea beetle)	TF[b]	A.c.	52

[a] Abbreviations: TF, tall fescue *(F. arundinacea)*; PRG, perennial ryegrass *(L. perenne)*; CF, Chewings fescue *(F. rubra* subsp. *commutata)*; HF, hard fescue *(F. longifolia)*; A.c., *A. coenophialum*; A.l., *A. lolii*; E.t., *E. typhina*.

[b] Based on field observations.

[c] M. Siegel, unpublished data.

Clavicipitaceae produce, or induce the plant to produce, auxinlike plant-growth regulators and/or plant growth inhibitors, or they may alter hormone metabolism. These are distinct possibilities when one considers that symptoms of infection by *E. typhina*, and other members of the Balansiae, include not only the inhibition of flowers and seed, but also enhanced plant growth and tillering, dwarfism, or deformation of the flag leaf (5, 17, 24, 37, 60, 110). Auxinlike indole compounds have been isolated in vitro from cultures of *Balansia epichloe* (79). The production of these compounds may reflect an alteration of nitrogen metabolism and assimilation in the infected plant (5).

TOLERANCE TO INSECT HERBIVORES Perhaps the most striking difference between *Acremonium*-infected and noninfected *Festuca* and *Lolium* species of grasses is the resistance by infected grasses to attack by insects (Table 1). It is now known that *Acremonium*-infected grasses produce an array of chemicals that have a wide range of biological activity (5, 10, 14, 82, 97).

The compounds responsible for insect (Argentine stem weevil, *Listronotus bonariensis*) resistance in *A. lolii*–infected perennial ryegrass have been identified as a peramine alkaloid (89) and the lolitrem neurotoxins (84). Peramine (89, 90), or a peraminelike compound (85), is made in culture by *A. lolii*.

The ergopeptide alkaloids (predominately ergovaline) isolated from *A. coenophialum*–infected tall fescue (67, 120) have not been reported active against insects. However, clavine ergot alkaloids (ergotamine and ergonovine) do have insecticidal properties (D. L. Dahlman, personal communication). The pyrrolizidine alkaloids (*N*-formyl and *N*-acetyl loline) produced in *A. coenophialum*–infected tall fescue (13, 15, 43, 50, 51) have also been reported responsible for insect resistance (45, 47). These alkaloids have not been recovered from either mycelium grown in culture or viable fungal mats isolated from enzyme-digested seed (L. P. Bush, personal communication). A steroid tetraenone metabolite (ergosta-4,6,8-(14), 22-tetraen-3-one), biologically active in brine shrimp and chick embryo bioassays, has been isolated from infected tall fescue and cultures of *A. coenophialum* (9, 21). This compound has not been tested for activity against insects.

Techniques now exist for producing synthetic grass–endophyte complexes. This synthesis involves the transfer of different species of fungi to host grasses and grasses not known to be hosts by inoculation of one-week-old seedlings (via wounding of the meristem) (56), by inoculation of callus tissue (46), and by plant breeding (maternal-line selection) (29, 30). These techniques should assist in further characterization of the grass–endophyte relationship and biological activity of synthesized chemicals.

One may assume that if biologically active chemicals are of fungal origin, they could be produced in any naturally occurring as well as in synthetic grass–endophyte combinations. Conversely, if compounds are only produced in conjunction with certain grass–fungus interactions, then a greater degree of specificity of synthesis would be expected. Peramine is not only produced in *A. lolii*–infected perennial ryegrass, but in *A. coenophialum* and *E. typhina* naturally infected grasses as well (M. R. Siegel, unpublished data). In addition, peramine was found in two synthetic grass–endophyte combinations: an *A. lolii*–infected tall fescue and *A. coenophialum*–infected perennial ryegrass. There appears to be a much greater specificity of synthesis of the loline alkaloids in infected grasses. Synthesis occurred only in *A. coenophialum*–infected tall fescue and in the synthetic *A. coenophialum*–infected perennial ryegrass. These alkaloids could not be recovered from tall fescue artificially infected with *E. typhina, A. lolii,* or *Phialophora*-like endophytes. Loline alkaloids have been reported in *L. temulentum* (19);

however, it is not known whether the plant material was infected with the *Acremonium*-like species that has been isolated from this grass (57). These data still do not explain whether the host, fungus, or both are responsible for synthesis of the loline alkaloids. It has yet to be determined whether the ergot alkaloids and lolitrems can be produced by other fungi in their natural hosts or by the fungi in nonhost grasses.

The ability of endophyte-infected plants to produce biologically active compounds under field conditions may depend on the location and concentration of the endophytes in the plant. Synthesis of these compounds may depend on factors that affect plant and fungal growth. These include internal and external nutritional conditions and the external environment (light, water, and temperature). The fungus-produced toxins peramine (90), ergot alkaloids (5, 67, 81), and lolitrems (33, 34) (classified here as being of fungal origin because of their similarity to other mycotoxins) are found in relatively low concentrations (0.3–22 μg/g plant dry weight) in infected plants. On the other hand, the loline alkaloids, presumed to be of plant origin, are found in concentrations as high as 8,253 μg/g plant dry weight (50).

The distribution and concentration of the chemicals within the plant vary with the compound, the location of the endophyte in the plant, and the season. There is little information on peramine, other than that concentrations of 1 μg/g plant fresh weight were recovered from *A. lolii*-infected plants (89). Leaf sheaths and blades of *A. lolii*-infected perennial ryegrass contained lolitrems at concentrations as high as 4.9 and 0.37 μg/g plant dry weight, respectively (33). The amount of *A. lolii* mycelium in infected plants has been positively correlated with resistance to Argentine stem weevil and with the time of year when ryegrass staggers occurs (11, 27, 82, 83).

Total ergot alkaloid concentrations in leaf sheaths and blades of *A. coenophialum*-infected tall fescue plants were 22 and 3.5 μg/g plant dry weight, respectively (5, 67). Total ergot alkaloids increased 2.5-fold in infected tall fescue following application of nitrogen fertilizers (67). Furthermore, added nitrogen was associated with increased growth of endophyte in the plant and increased symptoms of animal toxicoses (67). High levels of loline alkaloids have been found in the same parts of the plant where high densities of the fungus are known to exist (13, 43, 50, 51). In greenhouse studies, the fungus concentration in the plant did not increase during initial growth, but increased in first and second regrowth tissues (13). Loline alkaloid concentrations followed the same pattern, except after the second harvest, when concentrations in the blade (where there was little fungus) nearly equalled those in the stem. This finding suggests that these alkaloids, or the signal for their synthesis, are translocated to the leaf blades, and hence, regrowth tissue in the field may contain large amounts of alkaloids. In greenhouse experiments, loline alkaloids increased 2–3-fold in plants subjected to moderate-to-severe water stress (51). However, in contrast to the ergot alkaloids, increased application of nitrogen did not increase levels of loline alkaloids (51). Concentrations of loline alkaloids were monitored in tall fescue during a three-year steer-grazing study (103). They generally rose to their highest concentrations in August, and then remained constant until late fall. However, concentrations varied greatly from year to year, suggesting an effect of environment on alkaloid concentration in the plant.

There is little information on the effect of concentration of chemicals in infected plants and insect toxicity. Using the large Milkweed bug *(Oncopeltus fasciatus)* bioassay (47), D. L. Dahlman (personal communication) has found LD_{50} concentrations of ca 8 μg/ml for *N*-formyl loline, ergotamine, and ergonovine. Agar-plate bioassays of peramine with Argentine stem weevil indicated that feeding deterrence occurred at 10 μg/ml (89). No feeding deterrence occurred with lolitrems, but after 24-days feeding on 5 μg/ml, larval weights were reduced and 40% mortality occurred (84).

TOLERANCE TO GRAZING HERBIVORES In the section on animal toxicoses, we discussed the relationship between endophyte-infected grasses and animal maladies. One of the direct effects on animals grazing endophyte-infected grasses is a reduction in average daily weight gains. Of equal importance is the observation that infected tall fescue is tolerant to overgrazing by livestock (J. A. Boling, personal communication). The lack of overgrazing of the infected cultivars may be due to better growth of the plants and differences in agronomic factors between the infected and noninfected cultivars. More likely, it is due to the fact that animals feeding on infected perennial ryegrass and tall fescue consume substantially less forage than those feeding on noninfected grass (26, 102).

DISEASE RESISTANCE The last of the benefits of endophyte-infected grasses is the potential for disease resistance. White & Cole (113, 114) and M. R. Siegel & G. C. M. Latch (unpublished data) have demonstrated that isolates of *A. lolii, A. coenophialum,* unidentified *Acremonium* sp., *Phialophora*-like sp., and *E. typhina* from various grass spp. produced antibiosis against a range of fungal plant pathogens and saprophytic fungi in culture. The amount of antifungal activity and the spectrum of activity were found to be dependent on the isolate. Resistance to diseases in pasture or turf *Festuca* and *Lolium* spp. has not been reported.

Speculations on the Origins of Grass Endophytes

The recent origins of *A. coenophialum* and *A. lolii*–infected pasture and turf grasses have been discussed elsewhere (10, 96, 97). It is clear that Europe is the origin of these infected cultivated grasses. However, the determination that endophytes not only exist in native *Festuca* and *Lolium* species (112, 114, 116), but in large numbers of species in the Poaceae (111, 115, 117) as well, raises important questions concerning the recent origins of these endophytes and also the evolutionary relationships between grasses and endophytes. Because of the wide geographic distribution of cultivated and native grasses infected with endophytes, White (111) has suggested that the vegetative phases of these fungi are, or have evolved from, species of the ascomycetous genus *Epichloe*. Mycological and serological data (49, 73) and evolutionary concepts of biotrophy (63) give some support to this hypothesis.

Lewis (63) considers the biotrophic habit of great antiquity and believes that the basic features of the evolution of parasitism and mutualism within biotrophy occurred at least 400 million years ago. The evolution from parasitism to mutualism therefore depends on the capacity of the partners to avoid or tolerate environmental stresses, as well as the obvious capacity for two-way cycling of nutrients and other prerequisites of biotrophy previously discussed. As was pointed out earlier, *E. typhina* appears to be in the process of evolution, as indicated by the various degrees of symbiosis that exist between different isolates and their hosts. The end result of this evolutionary process would then be fungi like the *Acremonium* spp., which act as mutualistic symbionts.

USE OF ENDOPHYTE-INFECTED AND NONINFECTED GRASSES

Once specific aspects of the nature of the host–fungus interaction were recognized, then the advantages of both infected and noninfected grasses were realized. The fact that the grass–endophyte relationship could be manipulated to remove or introduce fungi into different cultivars of grasses only served to accelerate their use. However, it is now apparent from a better understanding of the grass–fungus relationship that whereas some uses of endophyte-free cultivars are desirable, they may not be practical. Instead, further manipulations of infected grasses may be necessary to realize the full potential of improved productivity from animals grazing infested pastures.

Use of Endophyte-Free Cultivars

As soon as it was recognized that endophyte-infected grasses were responsible for animal toxicoses, it was suggested that removal of the fungi would lead to an alleviation of symptoms and improved animal productivity (9, 27). Eradication of *A. lolii* and *A. coenophialum* from perennial ryegrass and tall fescue cultivars, respectively, was accomplished by killing the fungi in plants or seed by storage, heat, or chemical treatments.

Some of the endophyte-free tall fescue cultivars were clearly superior to infected cultivars from the standpoint of improved animal production (93, 102). However, the endophyte-free perennial ryegrass cultivars used in pastures in New Zealand were susceptible to insect predation and drought, and often did not survive (11, 82). Whether the same phenomenon will occur with the newly released endophyte-free tall fescue cultivars is unknown and is a topic of considerable speculation.

What is unclear is whether the superior character of tall fescue is due to the endophyte-grass complex or, in part, to the normal genetic complement of the plant. Consequently, it may be necessary to sow large areas of endophyte-free pastures before an appropriate evaluation of survival can be made.

Use of Endophyte-Infected Grasses

The confirmation of insect resistance, enhanced growth, and persistence of endophyte-infected grasses has led grass breeders to develop, by maternal line selection, naturally infected cultivars for turf (29–31). Seed of infected grasses used in turf will require special storage facilities and labeling to guarantee the viability of the endophytes.

Another approach to the use of endophyte-infected grasses would be to introduce specific fungal isolates, via wounding of seedlings, into host or nonhost grasses (synthetic grass–endophyte combinations). Assuming that the compounds toxic to animals are different from those that affect insects, it should be possible to develop synthetic combinations of host and endophyte suitable for turf and pasture. Insect deterrent compounds would be present in both turf and pasture cultivars. Turf grasses could still contain compounds toxic to animals, but pasture grasses would have little or none (10, 83, 89). Endophytes in these synthetic combinations could be naturally occurring biotypes, if suitable ones were available, or they could be genetically modified. All endophytes must, of course, be seed transmitted.

We have made a number of positive, but unproven, assumptions to support the use of modified endophyte-infected pasture grasses. We already know that the grass-endophyte system in some cases may contain more than one chemical responsible for insect resistance. This fact raises the question of what happens to insect resistance when one of several toxic compounds is not present in the modified synthetic combinations. It is obvious that it will be necessary to identify all the biologically active compounds and their spectrum of activity in plants, insects, and animals before new synthetic grass–endophyte cultivars are released for pasture use.

CONCLUDING REMARKS

Through interdisciplinary research, plant pathologists, chemists, agronomists, entomologists, and animal scientists have come to better understand a unique grass–fungal endophyte relationship. It is now clear that in certain grass–fungus complexes, mutualistic relationships result in benefits to both partners. Understanding this relationship has resulted in the development and release of both infected and noninfected cultivars for use in pasture and turf.

In order to ensure the proper use of these newly developed cultivars, interdisciplinary research must continue into the nature of the grass–endophyte relationship. Questions of interest and importance that need to be answered include the mechanisms of synthesis and the spectrum of activity of biologically active compounds; the interaction of external and internal stimuli on synthesis of these chemicals and on growth of the endophytes and plants; biochemical mechanisms of pest resistance and animal toxicoses; and the nature of biotrophy and how it relates to the ecological fitness and survival of the endophytes and grass hosts.

The widespread distribution of fungal endophytes in grasses and the potentially mutualistic nature of the relationships may suggest an importance that approaches other plant–microorganism complexes such as those that involve the mycorrhizal fungi and nitrogen-fixing bacteria. The improvement of grasses used for pasture, turf, and conservation are long-term goals of plant breeders; a fuller understanding of grass–endophyte relationships will aid in fulfilling these goals.

ACKNOWLEDGMENT

We wish to thank many authors for providing unpublished information and copies of manuscripts in press. Research in the senior author's laboratory was supported, in part, by USDA Competitive Research Grant 85-CRC-1-1533. This chapter is Kentucky Agricultural Experiment Station Journal Series Paper 87-11-9.

Literature Cited

1. Ahmad, S., Govindarajan, S., Funk, C. R., Johnson-Cicalese, J. M. 1985. Fatality of house crickets on perennial ryegrass infected with a fungal endophyte. *Entomol. Exp. Appl.* 39:183–90
2. Ahmad, S., Johnson-Cicalese, J. M., Dickson, W. K., Funk, C. R. 1986. Endophyte-enhanced resistance in perennial ryegrass to the bluegrass bill-bug *Sphenophorus parvulus. Entomol. Exp. Appl.* 41:3–10
3. Bacon, C. W. 1985. A chemically defined medium for the growth and synthesis of ergot alkaloids by species of *Balansia. Mycologia* 77:418–23
4. Bacon, C. W., Hinton, D. 1983. Biology of the endophyte of fescue: Ultrastructural analysis and physiological relationships. In *Proc. Forage & Turfgrass Endophyte Workshop,* pp. 19–28. Corvallis, Ore: Oregon State Univ.
5. Bacon, C. W., Lyons, P. C., Porter, J. K., Robbins, J. D. 1986. Ergot toxicity from endophyte-infected grasses: A review. *Agron. J.* 78:106–16
6. Bacon, C. W., Porter, J. K., Robbins, J. D. 1975. Toxicity and occurrence of *Balansia* on grasses from toxic fesuce pastures. *Appl. Microbiol.* 29:553–56
7. Bacon, C. W., Porter, J. K., Robbins, J. D. 1979. Laboratory production of crgot alkaloids by species of *Balansia. J. Gen. Microbiol.* 113:119–26
8. Bacon, C. W., Porter, J. K., Robbins, J. D. 1981. Ergot alkaloid biosynthesis by

isolates of *Balansia epichloe* and *B. henningsiana*. *Can. J. Bot.* 59:2534–38

9. Bacon, C. W., Porter, J. K., Robbins, J. D., Luttrell, E. S. 1977. *Epichloe typhina* from toxic tall fescue grasses. *Appl. Environ. Microbiol.* 34:576–81
10. Bacon, C. W., Siegel, M. R. 1987. The endophyte of tall fescue. *J. Prod. Agric.* In press
11. Barker, G. M., Prestidge, R. A., Pottinger, R. P. 1985. Strategies for Argentine stem weevil control: Effects of drought and endophyte. *Proc. N. Z. Grassl. Assoc.* 47:107–14
12. Bolt, D. J., Bond, J., Lynch, G. P., Elsasser, T. 1982. Concentrations of PRL, LH, FSH, GH and TSH in plasma and pituitary of ewes grazing tall fescue and orchardgrass pastures. *J. Anim. Sci.* 55(Suppl. 1):4(Abstr.)
13. Burhan, W., Bush, L. P. 1987. Development of the endophyte *Acremonium coenophialum* and accumulation of N-acetyl and N-formyl loline in tall fescue (*Festuca arundinacea* Schreb.). *Crop Sci.* In press
14. Bush, L. P., Burrus, P. B. II. 1987. Tall fescue quality as affected by *Acremonium coenophialum*. *J. Prod. Agric.* In press
15. Bush, L. P., Cornelius, P. L., Buckner, R. C., Varney, D. R., Chapman, R. A., et al. 1982. Association of N-acetyl loline and N-formyl loline with *Epichloe typhina* in tall fescue. *Crop Sci.* 22:941–43
16. Clark, E. M., White, J. F., Patterson, R. M. 1983. Improved histochemical techniques for the detection of *Acremonium coenophialum* in tall fescue and methods of in vitro culture of the fungus. *J. Microbiol. Methods* 1:149–55
17. Clay, K. 1984. The effect of the fungus *Atkinsonella hypoxylon* (Clavicipitaceae) on the reproductive system and demography of the grass *Danthonia spicata*. *New Phytol.* 98:165–75
18. Cole, G. T., White, J. F. 1985. Fungal endophytes of forage grasses. *Proc. Indian Acad. Sci. (Plant Sci.)* 94:129–36
19. Culvenor, C. C. 1973. Alkaloids. In *Chemistry and Biochemistry of Herbage*, ed. G. W. Butler, R. W. Bailey, 1:375–446. London/New York: Academic
20. Davis, N. D., Clark, E. M., Schrey, K. A., Diener, U. L. 1986. In vitro growth of *Acremonium coenophialum*, an endophyte of toxic tall fescue grass. *Appl. Environ. Microbiol.* 52:888–91
21. Davis, N. D., Cole, R. J., Dorner, J. W., Weete, J. D., Backman, P. A., et al. 1986. Steroid metabolites of *Acremonium coenophialum*, an endophyte of tall fescue. *J. Agric. Food Chem.* 34:105–8
22. de Bary, A. 1887. *Comparative Morphology and Biology of the Fungi, Mycetozoa and Bacteria*. Oxford: Claredon. (English translation)
23. di Menna, M. E., Waller, J. E. 1986. Visual assessment of seasonal changes in amount of mycelium of *Acremonium loliae* in leaf sheaths of perennial ryegrass. *N. Z. J. Agric. Res.* 29:111–16
24. Diehl, W. W. 1950. *Balansia* and the *Balansiae* in America. *Agric. Monogr. 4*. Washington, DC: US Dep. Agric.
25. Fineran, B. A., Harvey, I. C., Ingerfeld, M. 1983. Unusual crystalloids and aggregates of tubules in the *Lolium* endophyte of ryegrass leaf sheaths. *Protoplasma* 117:17–23
26. Fletcher, L. R., Barrell, G. K. 1984. Reduced liveweight gains and serum prolactin levels in hoggets grazing ryegrasses containing *Lolium* endophyte. *N. Z. Vet. J.* 32:139–40
27. Fletcher, L. R., Harvey, I. C. 1981. An association of a *Lolium* endophyte with ryegrass staggers. *N. Z. Vet. J.* 29:185–86
28. Freeman, E. M. 1903. The seed-fungus of *Lolium temulentum* L., the darnel. *Philos. Trans. R. Soc. London Ser. B* 196:1–27
29. Funk, C. R., Halisky, P. M., Ahmad, S. Hurley, R. H. 1985. How endophytes modify turfgrass performance and response to insect pests in turfgrass breeding and evaluation trials. In *Proc. 5th Int. Turf Res. Conf. Avignon*, ed. F. Lemaire, pp. 137–45. Versailles: INRA
30. Funk, C. R., Halisky, P. M., Hurley, R. H. 1983. Implications of endophytic fungi in breeding for insect resistance. See Ref. 4, pp. 67–75
31. Funk, C. R., Halisky, P. M., Johnson, M. C., Siegel, M. R., Stewart, A. V., et al. 1983. An endophytic fungus and resistance to sod webworms: Association in *Lolium perenne* L. *Bio/Technol.* 1:189–91
32. Gallagher, R. T., Hawkes, A. D. 1985. Estimation of neurotoxin levels in perennial ryegrass by mouse bioassay. *N. Z. J. Agric. Res.* 28:427–31
33. Gallagher, R. T., Hawkes, A. D., Stewart, J. M. 1985. Rapid determination of the neurotoxin lolitrem B in perennial ryegrass by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr.* 321:217–26

34. Gallagher, R. T., Hawkes, A. D., Steyn, P. S., Vleggaar, R. 1984. Tremorgenic neurotoxins from perennial ryegrass causing ryegrass staggers disorder of livestock: Structure and elucidation of lolitrem B. *J. Chem. Soc. Chem. Commun.* 614–16
35. Garner, G. B., Cornell, C. N. 1978. Fescue foot in cattle. In *Mycotoxic Fungi, Mycotoxins, Mycotoxicoses: An Encyclopedic Handbook,* ed. T. D. Wyllie, L. G. Morehouse, 2:45–62. New York: Dekker
36. Gay, N., Boling, J. A., Miksch, D. 1986. Effects of fescue pastures on cow-calf performance. *J. Anim. Sci.* 63 (Suppl. 1):73(Abstr.)
37. Harberd, D. J. 1961. Note on choke disease in *Festuca rubra. Scottish Plant Breeding Station Report,* pp. 47–51. Edinburgh: Blackwood
38. Hardy, T. N., Clay, K., Hammond, A. M. Jr. 1985. Fall armyworm (Lepidoptera: Noctuidae): A laboratory bioassay and larval preference study for the fungal endophyte of perennial ryegrass. *J. Econ. Entomol.* 78:571–75
39. Hardy, T. N., Clay, K., Hammond, A. M. Jr. 1986. Leaf age and related factors affecting endophyte-mediated resistance to fall armyworm (Lepidoptera: Noctuidae) in tall fescue. *Environ. Entomol.* 15:1083–89
40. Hemken, R. W., Jackson, J. A. Jr., Boling, J. A. 1984. Toxic factors in tall fescue. *J. Anim. Sci.* 58:1011–16
41. Hinton, D. M., Bacon, W. C. 1985. The distribution and ultrastructure of the endophyte of toxic tall fescue. *Can. J. Bot.* 63:36–42
42. Hurley, W. L., Convey, E. M., Leung, K., Edgerton, L. A., Hemken, R. W. 1981. Bovine prolactin, TSH, T_4 and T_3 concentrations as affected by tall fescue summer toxicosis and temperature. *J. Anim. Sci.* 51:374–79
43. Jackson, J. A., Hemken, R. W., Boling, J. A., Harmon, R. J., Buckner, R. C., Bush, L. P. 1984. Loline alkaloids in tall fescue hay and seed and their relationship to summer fescue toxicosis in cattle. *Dairy Sci.* 67:104–9
44. Johnson, M. C., Anderson, R. L., Kryscio, R. J., Siegel, M. R. 1983. Sampling procedures for determining endophyte in tall fescue seed lots by ELISA. *Phytopathology* 73:1406–9
45. Johnson, M. C., Bush, L. P., Siegel, M. R. 1986. Aphid feeding deterrence associated with pyrrolizidine alkaloids present in endophyte-infected tall fescue. *Phytopathology* 76:1057 (Abstr.)
46. Johnson, M. C., Bush, L. P., Siegel, M. R. 1986. Infection of tall fescue with *Acremonium coenophialum* by means of callus culture. *Plant Dis.* 70:380–82
47. Johnson, M. C., Dahlman, D. L., Siegel, M. R., Bush, L. P., Latch, G. C. M., et al. 1985. Insect feeding deterrents in endophyte-infected tall fescue. *Appl. Environ. Microbiol.* 49:568–71
48. Johnson, M. C., Pirone, T. P., Siegel, M. R., Varney, D. R. 1982. Detection of *Epichloe typhina* in tall fescue by means of enzyme-linked immunosorbent assay. *Phytopathology* 72:647–50
49. Johnson, M. C., Siegel, M. R., Schmidt, B. A. 1985. Serological reactivities of endophytic fungi from tall fescue and perennial ryegrass and of *Epichloe typhina. Plant Dis.* 69:200–2
50. Jones, T. A., Buckner, R. C., Burrus, P. B. II, Bush, L. P. 1983. Accumulation of pyrrolizidine alkaloids in benomyl-treated tall fescue parents and their untreated progenies. *Crop Sci.* 23:1135–40
51. Kennedy, C. W., Bush, L. P. 1983. Effect of environmental and management factors on the accumulation of N-acetyl and N-formyl loline alkaloids in tall fescue. *Crop Sci.* 23:547–52
52. Kirfman, G. W., Brandenburg, R. L., Garner, G. B. 1986. Relationship between insect abundance and endophyte infestation level in tall fescue in Missouri. *J. Kans. Entomol. Soc.* 59:552–54
53. Kulkarni, R. K., Nielsen, B. D. 1986. Nutritional requirements for growth of a fungus endophyte of tall fescue. *Mycologia* 78:781–86
54. Latch, G. C. M. 1983. Incidence of endophytes in seedlines and their control with fungicides. *Proc. N. Z. Grassl. Assoc.* 44:251–53
55. Latch, G. C. M., Christensen, M. J. 1982. Ryegrass endophyte, incidence, and control. *N. Z. J. Agric. Res.* 25:443–48
56. Latch, G. C. M., Christensen, M. J. 1985. Artificial infection of grasses with endophytes. *Ann. Appl. Biol.* 107:17–24
57. Latch, G. C. M., Christensen, M. J. 1987. Endophytes of annual and hybrid ryegrasses. *N. Z. J. Agric. Res.* In press
58. Latch, G. C. M., Christensen, M. J., Gaynor, D. L. 1985. Aphid detection of endophyte infection in tall fescue. *N. Z. J. Agric. Res.* 28:129–32
59. Latch, G. C. M., Christensen, M. J., Samuels, G. J. 1984. Five endophytes of

Lolium and *Festuca* in New Zealand. *Mycotaxon* 20:535–50

60. Latch, G. C. M., Hunt, W. F., Musgrave, D. R. 1985. Endophytic fungi affect growth of perennial ryegrass. *N. Z. J. Agric. Res.* 28:165–68
61. Latch, G. C. M., Potter, L. R., Tyler, B. F. 1987. Incidence of endophytes in seeds from collections of *Lolium* and *Festuca* species. *Ann. Appl. Biol.* In press
62. Lewis, D. H. 1973. Concepts in fungal nutrition and the origin of biotrophy. *Biol. Rev.* 48:261–78
63. Lewis, D. H. 1974. Micro-organisms and plants: The evolution of parasitism and mutualism. *24th Symp. Soc. Gen. Microbiol*, pp. 367–92. England: Cambridge Univ. Press
64. Lindau, G. 1904. Über das Vorkommen des Pilzes des Taumellolchs in altägyptischen Sämen. *Sitzungsber. K. Preuss. Akad. Wissen*, pp. 1031–36
65. Lloyd, A. B. 1959. The endophytic fungus of perennial ryegrass. *N. Z. J. Agric. Res.* 2:1187–94
66. Luttrell, E. S., Bacon, C. W. 1977. Classification of *Myriogenospora* in the Clavicipitaceae. *Can. J. Bot.* 55:2090–97
67. Lyons, P. C., Plattner, R. D., Bacon, C. W. 1986. Occurrence of peptide and clavine ergot alkaloids in tall fescue. *Science* 232:487–89
68. Mantle, P. G. 1978. Ergotism in cattle. See Ref. 35, pp. 145–51
69. McLennan, E. 1920. The endophytic fungus of *Lolium*, I. *Proc. R. Soc. Victoria* 32:252–301
70. Morgan-Jones, G., Gams, W. 1982. Notes on Hyphomycetes, XLI. An endophyte of *Festuca arundinacea* and the anamorph of *Epichloe typhina*, new taxa in one of two new sections of *Acremonium*. *Mycotaxon* 15:311–18
71. Mortimer, P. H., di Menna, M. E. 1985. Interactions of *Lolium* endophyte on pasture production and perennial ryegrass staggers disease. In *Trichothecenes and Other Mycotoxins*, ed. J. Lacey, pp. 149–58. Chichester/New York: Wiley 571 pp.
72. Musgrave, D. R. 1984. Detection of an endophytic fungus of *Lolium perenne* using enzyme-linked immunosorbent assay (ELISA). *N. Z. J. Agric. Res.* 27:283–88
73. Musgrave, D. R., Fletcher, L. R. 1986. Optimisation and characterisation of enzyme-linked immunosorbent assay (ELISA) for the detection of the *Acremonium loliae* endophyte in *Lolium perenne*. *N. Z. J. Agric. Res.* 29:117–20
74. Musgrave, D. R., Grose, T. A., Latch, G. C. M., Christensen, M. J. 1986. Purification and characterisation of the antigens of endophytic fungi isolated from *Lolium perenne* and *Festuca arundinacea* in New Zealand. *N. Z. J. Agric. Res.* 29:121–28
75. Neill, J. C. 1940. The endophyte of ryegrass *(Lolium perenne)*. *N. Z. J. Sci. Technol. Sec. A* 21:280–91
76. Nestler, A. 1898. Ueber einen in der Frucht von *Lolium temulentum* L. vorkommenden Pilz. *Ber. Dtsch Bot. Ges.* 16:207–14
77. Peterson, A. J., Bass, J. J., Byford, M. J. 1984. Decreased plasma testosterone concentrations and weight gain in young bulls affected by ryegrass staggers. *N. Z. Vet. J.* 32:36–37
78. Philipson, M. N., Christey, M. C. 1986. The relationship of host and endophyte during flowering, seed formation, and germination of *Lolium perenne*. *N. Z. J. Bot.* 24:125–34
79. Porter, J. K., Bacon, C. W., Cutler, H. G., Arrendale, R. F., Robbins, J. D. 1985. In vitro auxin production by *Balansia epichloe*. *Phytochemistry* 24: 1429–31
80. Porter, J. K., Bacon, C. W., Robbins, J. D. 1979. Ergosine, ergosinine, and chanoclavine I from *Epichloe typhina*. *J. Agric. Food Chem.* 27:595–98
81. Porter, J. K., Bacon, C. W., Robbins, J. D., Betowski, D. 1981. Ergot alkaloid identification in Clavicipitaceae systemic fungi of pasture grasses. *J. Agric. Food Chem.* 29:653–58
82. Pottinger, R. P., Barker, G. M., Prestidge, R. A. 1985. A review of the relationships between endophytic fungi of grasses (*Acremonium* spp.) and Argentine stem weevil (*Listronotus bonariensis* Kuschel). *Proc. 4th Australas. Conf. Grassl. Invert. Ecol. Lincoln Coll., Canterbury*, pp. 322–31
83. Prestidge, R. A., di Menna, M. E., van der Zijpp, S. 1985. Minimum *Acremonium loliae* levels needed to maintain Argentine stem weevil resistance in infected ryegrasses and in cultures. See Ref. 82, pp. 332–37
84. Prestidge, R. A., Gallagher, R. T. 1985. Lolitrem B—A stem weevil toxin isolated from *Acremonium*-infected ryegrass. *Proc. N. Z. Weed Pest Control Conf.* 38:38–40
85. Prestidge, R. A., Lauren, D. R., van der Zijpp, S. G., di Menna, M. E. 1985. Isolation of feeding deterrents to Argentine stem weevil in cultures of en-

dophytes of perennial ryegrass and tall fescue. *N. Z. J. Agric. Res.* 28:87–92

86. Prestidge, R. A., Pottinger, R. P., Barker, G. M. 1982. An association of *Lolium* endophyte with ryegrass resistance to Argentine stem weevil. *Proc. N. Z. Weed Pest Control Conf.* 35:119–22
87. Read, J. C., Camp, B. J. 1986. The effect of the fungal endophyte *Acremonium coenophialum* in tall fescue on animal performance, toxicity, and stand maintenance. *Agron. J.* 78:848–50
88. Rolston, M. P., Hare, M. D., Moore, K. K., Christensen, M. J. 1986. *Lolium* endophyte fungi viability with storage at different seed moisture and temperatures. *N. Z. J. Exp. Agric.* 14:297–300
89. Rowan, D. D., Gaynor, D. L. 1986. Isolation of feeding deterrents against Argentine stem weevil from ryegrass infected with the endophyte *Acremonium loliae. J. Chem. Ecol.* 12:647–58
90. Rowan, D. D., Hunt, M. B., Gaynor, D. L. 1986. Peramine, a novel insect feeding deterrent from ryegrass infected with the endophyte *Acremonium loliae. J. Chem. Soc. Chem. Commun.* 935–36
91. Rykard, D. M., Bacon, C. W., Luttrell, E. S. 1985. Host relations of *Myriogenospora atramentosa* and *Balansia epichloe* (Clavicipitaceae). *Phytopathology* 75:950–56
92. Sampson, K. 1933. The systemic infection of grasses by *Epichloe typhina* (Pers.) Tul. *Trans. Br. Mycol. Soc.* 18:30–47
93. Schmidt, S. P., Hoveland, C. S., Clark, E. M., Davis, N. D., Smith, L. A., et al. 1982. Association of an endophytic fungus with fescue toxicity in steers fed Kentucky 31 tall fescue seed or hay. *J. Anim. Sci.* 55:1259–63
94. Scott, D. J. 1983. *Lolium* endophyte levels in commercial perennial ryegrass seed lines. *Proc. N. Z. Grassl. Assoc.* 44:254–56
95. Shelby, R. A., Dalrymple, L. W. 1987. Incidence and distribution of the tall fescue endophyte in the United States. *Plant Dis.* In press
96. Siegel, M. R., Johnson, M. C., Varney, D. R., Nesmith, W. C., Buckner, R. C., et al. 1984. A fungal endophyte in tall fescue: Incidence and dissemination. *Phytopathology* 74:932–37
97. Siegel, M. R., Latch, G. C. M., Johnson, M. C. 1985. *Acremonium* fungal endophytes of tall fescue and perennial ryegrass: significance and control. *Plant Dis.* 69:179–83
98. Siegel, M. R., Varney, D. R., Johnson, M. C., Nesmith, W. C., Buckner, R. C., et al. 1984. A fungal endophyte of tall fescue: Evaluation of control methods. *Phytopathology* 74:937–41
99. Smith, K. T., Bacon, C. W., Luttrell, E. S. 1985. Reciprocal translocation of carbohydrates between host and fungus in bahiagrass infected with *Myriogenospora atramentosa. Phytopathology* 75:407–11
100. Snell, W. H., Dick, E. A. 1971. *A Glossary of Mycology*. Cambridge, Mass: Harvard Univ. Press
101. Stewart, A. V. 1985. Perennial ryegrass seedling resistance to Argentine stem weevil. *N. Z. J. Agric. Res.* 28:403–7
102. Strahan, S. R., Hemken, R. W., Jackson, J. A. Jr., Buckner, R. C., Bush, L. P., Siegel, M. R. 1987. Performance of lactating dairy cows fed tall fescue forage. *J. Dairy Sci.* In press
103. Stuedemann, J. A., Rumsey, T. S., Bond, J., Wilkinson, S. R., Bush, L. P., et al. 1985. Association of blood cholesterol with occurrence of fat necrosis in cows and tall fescue summer toxicosis in steers. *Am. J. Vet. Res.* 46:1990–95
104. Varney, D. R., Ndefru, M., Jones, S. L., Siegel, M. R., Zavos, P. 1986. The effect of the fescue endophyte on the rat estrous cycle. *Assoc. Southeastern Biol. Bull.* 33:66(Abstr.)
105. Vogl, A. E. 1898. Mehl und die anderen Mehlprodukte der Cerealien und Leguminosen. *Nahrungsm. Unters. Hyg. Warenk.* 12:25–29
106. Walker, J. C. 1950. *Plant Pathology*. New York: McGraw-Hill
107. Wallner, B. M., Booth, N. H., Robbins, J. D., Bacon, C. W., Porter, J. K., et al. 1983. Effect of an endophytic fungus isolated from toxic pasture grass on serum prolactin concentrations in the lactating cow. *Am. J. Vet. Res.* 44:1317–22
108. Welty, R. E., Azevedo, M. D., Cook, K. L. 1986. Detecting viable *Acremonium* endophytes in leaf sheaths and meristems of tall fescue and perennial ryegrass. *Plant Dis.* 70:431–35
109. Welty, R. E., Milbrath, G. M., Faulkenberry, D., Azevedo, M. D., Meek, L., Hall, K. 1986. Endophyte detection in tall fescue seed by staining and ELISA. *Seed Sci. Technol.* 14:105–16
110. Western, J. H., Cavett, J. J. 1959. The choke disease of cocksfoot *(Dactylis glomerata)* caused by *Epichloe typhina* (Fr.) Tul. *Trans. Br. Mycol. Soc.* 42:298–307
111. White, J. F. Jr. 1987. The widespread

distribution of endophytes in the Poaceae. *Plant Dis.* In press

112. White, J. F. Jr., Cole, G. T. 1985. Endophyte-host associations in forage grasses. I. Distribution of fungal endophytes in some species of *Lolium* and *Festuca*. *Mycologia* 77:323–27
113. White, J. F. Jr., Cole, G. T. 1985. Endophyte-host associations in forage grasses. III. In vitro inhibition of fungi by *Acremonium coenophialum*. *Mycologia* 77:487–89
114. White, J. F. Jr., Cole, G. T. 1986. Endophyte-host associations in forage grasses. IV. The endophyte of *Festuca versuta*. *Mycologia* 78:102–7
115. White, J. F. Jr., Cole, G. T. 1986. Endophyte-host associations in forage grasses. V. Occurrence of fungal endophytes in certain species of *Bromus* and *Poa*. *Mycologia* 78:846–50
116. White, J. F. Jr., Cole, G. T., Morgan-Jones, G. 1987. Endophyte-host associations in forage grasses. VI. A new species of *Acremonium* isolated from *Festuca arizonica*. *Mycologia* 79:148–52
117. White, J. F. Jr., Morgan-Jones, G. 1987. Endophyte-host associations in forage grasses. VII. *Acremonium chisosum,* a new species isolated from *Stipa eminens* in Texas. *Mycotaxon* 28:179–89
118. Williams, M. J., Backman, P. A., Clark, E. M., White, J. F. 1984. Seed treatments for control of the tall fescue endophyte *Acremonium coenophialum*. *Plant Dis.* 68:49–52
119. Williams, M. J., Backman, P. A., Crawford, M. A., Schmidt, S. P., King, C. C. 1984. Chemical control of the tall fescue endophyte and its relationship to cattle performance. *N. Z. J. Exp. Agric.* 12:165–71
120. Yates, S. G., Plattner, R. D., Garner, G. B. 1985. Detection of ergopeptine alkaloids in endophyte infected, toxic Ky-31 tall fescue by mass spectrometry/mass spectrometry. *J. Agric. Food Chem.* 33:719–22
121. Zavos, P. M., Salim, B., Jackson, J. A. Jr., Varney, D. R., Siegel, M. R., Hemken, R. W. 1986. Effect of feeding tall fescue seed infected by endophytic fungus *(Acremonium coenophialum)* on reproductive performance in male rats. *Theriogenology* 25:281–90
122. Zavos, P. M., Varney, D. R., Jackson, J. A., Siegel, M. R., Bush, L. P., et al. 1987. Effect of feeding fungal endophyte *(Acremonium coenophialum)* infected tall fescue seed on reproductive performance in CD-1 mice through continuous breeding. *Theriogenology*. In press
123. Zavos, P. M., Varney, D. R., Siegel, M. R., Hemken, R. W., Jackson, J. A., et al. 1987. Effects of feeding endophyte-infected tall fescue seed on the reproductive performance in male and female CD-1 mice via combination crosses. *Theriogenology*. In press